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## Treatment response evaluation with 18F-FDG PET/CT and 18F-NaF PET/CT in multiple myeloma patients undergoing high-dose chemotherapy and autologous stem cell transplantation --Manuscript Draft--

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Abstract:	<p>The aim of this study was to assess the combined use of the radiotracers 18F-FDG and 18F-NaF in treatment response evaluation of a group of multiple myeloma (MM) patients undergoing high-dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) by means of static (whole-body) and dynamic PET/CT (dPET/CT). Patients and methods: 34 patients with primary, previously untreated MM scheduled for treatment with HDT followed by ASCT were enrolled in the study. All patients underwent PET/CT scanning with 18F-FDG and 18F-NaF before and after therapy. Treatment response by means of PET/CT was assessed according to the European Organization for Research and Treatment of Cancer (EORTC) 1999 criteria. The evaluation of dPET/CT studies was based on qualitative evaluation, semi-quantitative (SUV) calculation, and quantitative analysis based on 2-tissue compartment modelling and a non-compartmental approach leading to the extraction of fractal dimension (FD). Results: An analysis was possible in 29 patients: 3 with clinical complete response (CR) and 26 with non-CR (13 patients near complete response-nCR, 4 patients very good partial response-VGPR, 9 patients partial response-PR). After treatment, 18F-FDG PET/CT was negative in 14/29 patients and positive in 15/29 patients, showing a sensitivity of 57.5% and a specificity of 100%. According to the EORTC 1999 criteria, 18F-FDG PET/CT-based treatment response revealed CR in 14 patients (18F-FDG PET/CT CR), PR in 11 patients (18F-FDG PET/CT PR) and</p>

	<p>progressive disease in 4 patients (18F-FDG PET/CT PD). In terms of 18F-NaF PET/CT, 4/29 pts (13.8%) had a negative baseline scan, thus failed to depict MM. Regarding the patients, for which a direct lesion-to-lesion comparison was feasible, 18F-NaF PET/CT depicted 56 of the 129 18F-FDG positive lesions (43%). Follow-up 18F-NaF PET/CT showed persistence of 81.5% of the baseline 18F-NaF positive MM lesions after treatment, despite the fact that 64.7% of them had turned to 18F-FDG negative. Treatment response according to 18F-NaF PET/CT revealed CR in 1 patient (18F-NaF PET/CT CR), PR in 5 patients (18F-NaF PET/CT PR), SD in 12 patients (18F-NaF PET/CT SD), and PD in 7 patients (18F-NaF PET/CT PD). Dynamic 18F-FDG and 18F-NaF PET/CT studies showed that SUVaverage, SUVmax, as well as the kinetic parameters K1, influx and FD from reference bone marrow and skeleton responded to therapy with a significant decrease (<math>p &lt; 0.001</math>). Conclusion: 18F-FDG PET/CT demonstrated a sensitivity of 57.7% and a specificity of 100% in treatment response evaluation of MM. Despite its limited sensitivity, the performance of 18F-FDG PET/CT was satisfactory, given that 6/9 false negative patients in follow-up scans (66.7%) were clinically characterized as nCR, a disease stage with very low tumor mass. On the other hand, 18F-NaF PET/CT does not seem to add significantly to 18F-FDG PET/CT in treatment response evaluation of MM patients undergoing HDT and ASCT, at least shortly after therapy.</p>
<b>Response to Reviewers:</b>	

Dear Editor,

Please find below our response to the Reviewers' comments on our manuscript entitled "Treatment response evaluation with  $^{18}\text{F}$ -FDG PET/CT and  $^{18}\text{F}$ -NaF PET/CT in multiple myeloma patients undergoing high-dose chemotherapy and autologous stem cell transplantation"

by C. Sachpekidis, J. Hillengass, H. Goldschmidt, B. Wagner, U. Haberkorn, K. Kopka, A. Dimitrakopoulou-Strauss

**Reviewer #1:**

Adequate. No remarks

**Reviewer #2:**

Authors have made extensive modification on the original manuscript based on our comment. But two questions still remain to be answered.

1. Authors provided the results of PFS and OS in the revised manuscript in Table 1.

Data without appropriate analysis doesn't lead to convincing conclusions. Authors did not proceed to survival analysis due to lack of late follow-up data for all patients. Why don't authors proceed to progression-free survival analysis?

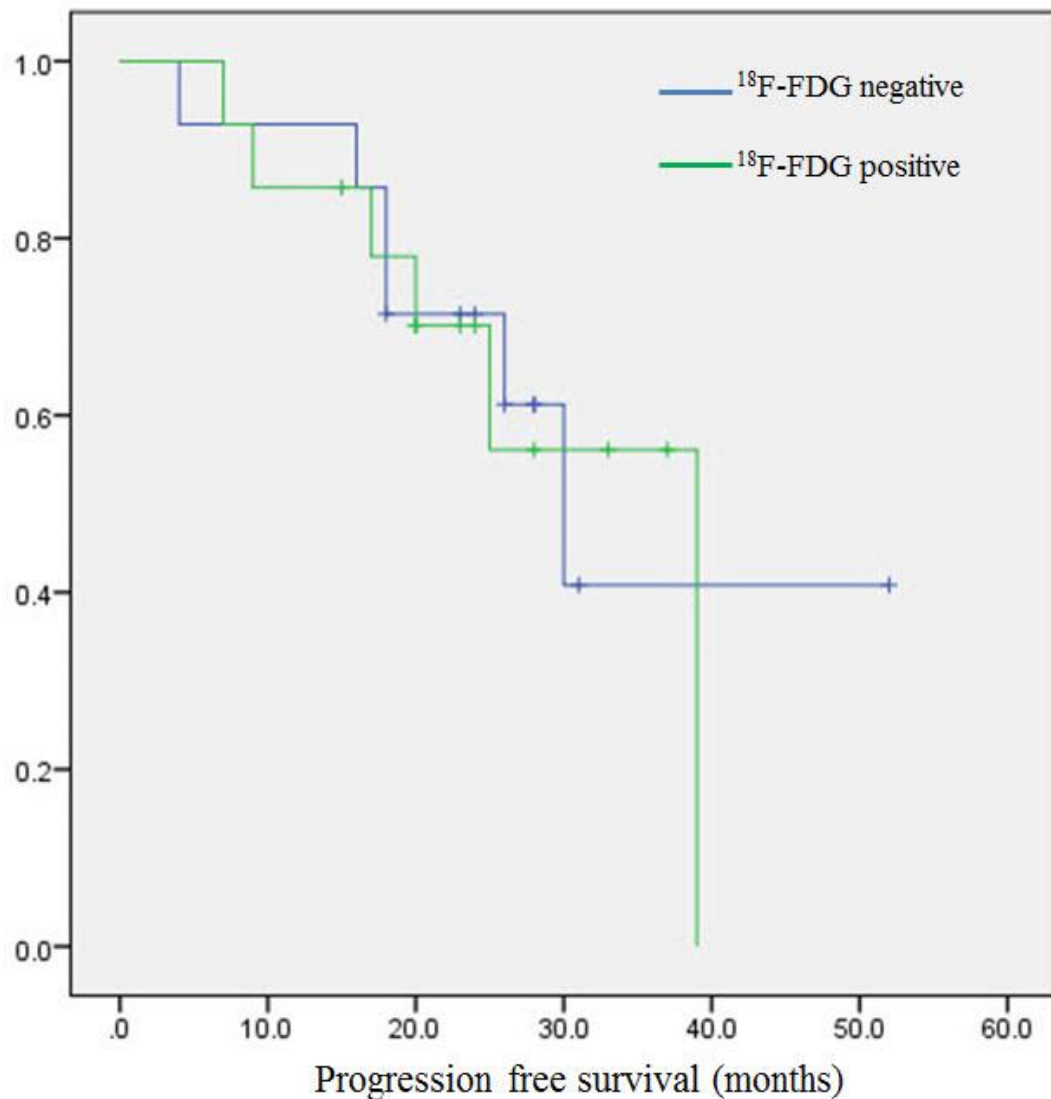
**Authors' response:** We proceeded to progression-free survival analysis for 28 patients, since, as already mentioned, one patient was lost to follow-up. By the time of writing 12 patients demonstrated progression. We dichotomized patients in PET/CT-positive (complete response) and PET/CT-negative (non-complete response) after therapy (follow-up scan). 6/12 patients demonstrated complete  $^{18}\text{F}$ -FDG response and 6/12 patients had non-complete  $^{18}\text{F}$ -FDG response. The results of Kaplan-Meier analysis and a graph are now presented in the Point to point discussion (please see below Table 1, Figure 1). No statistically significant difference in PFS was observed between the  $^{18}\text{F}$ -FDG-positive and  $^{18}\text{F}$ -FDG-negative patients. We would prefer not to include the results of this

analysis in the manuscript, since the number of events is still small. However, if the Reviewer insists, we could present them data as supplementary data.

Due to the fact that only one patient showed complete response in  $^{18}\text{F}$ -NaF PET/CT ( $^{18}\text{F}$ -NaF negative follow-up PET/CT), we did not perform similar dichotomization and survival analysis for this tracer.

	Median (months)	Mean (months)
follow-up $^{18}\text{F}$ -FDG negative	29.4	34
follow-up $^{18}\text{F}$ -FDG positive	39	30

**Table 1.** Mean and median PFS values of the 12 patients demonstrating progression, dichotomized according to the result of follow-up  $^{18}\text{F}$ -FDG PET/CT. The PFS difference between these two groups was not statistically significant (log-rank  $p=0.848$ ).



**Figure 1:** Kaplan-Meier plot of PFS. No statistically significant difference in PFS between patients with negative (blue curve) and positive (green curve) follow-up <sup>18</sup>F-FDG PET/CT (log-rank  $p = 0.848$ ).

2. I just want to know how to calculate the dosages of <sup>18</sup>F-FDG and <sup>18</sup>F-NaF. Table 2 is unnecessary. The dosages can be described with interval numbers.

**Authors' response:** Table 2 was removed and dosage ranges of both tracers are now provided in text (pg 5, para 2, ln 6-7). There was a maximum limit of 250 MBq for each PET exam, as defined by the federal radiation protection agency. The administered dose was not weight-dependent. We tried to apply as much

activity as possible with respect to the predefined upper limit. Nevertheless, due to technical reasons (e.g. delays in delivery of tracer), in very few cases relative low doses of tracer activity were administered (for example baseline  $^{18}\text{F}$ -FDG PET/CT of patient 13).

# Treatment response evaluation with $^{18}\text{F}$ -FDG PET/CT and $^{18}\text{F}$ -NaF PET/CT in multiple myeloma patients undergoing high-dose chemotherapy and autologous stem cell transplantation

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**Key Words:**  $^{18}\text{F}$ -FDG;  $^{18}\text{F}$ -NaF; PET/CT; high-dose chemotherapy; autologous stem cell transplantation; two-tissue compartment model

## ABSTRACT

The aim of this study was to assess the combined use of the radiotracers  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -NaF in treatment response evaluation of a group of multiple myeloma (MM) patients undergoing high-dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) by means of static (whole-body) and dynamic PET/CT (dPET/CT). **Patients and methods:** 34 patients with primary, previously untreated MM scheduled for treatment with HDT followed by ASCT were enrolled in the study. All patients underwent PET/CT scanning with  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -NaF before and after therapy. Treatment response by means of PET/CT was assessed according to the European Organization for Research and Treatment of Cancer (EORTC) 1999 criteria. The evaluation of dPET/CT studies was based on qualitative evaluation, semi-quantitative (SUV) calculation, and quantitative analysis based on 2-tissue compartment modelling and a non-compartmental approach leading to the extraction of fractal dimension (FD). **Results:** An analysis was possible in 29 patients: 3 with clinical complete response (CR) and 26 with non-CR (13 patients near complete response-nCR, 4 patients very good partial response-VGPR, 9 patients partial response-PR). After treatment,  $^{18}\text{F}$ -FDG PET/CT was negative in 14/29 patients and positive in 15/29 patients, showing a sensitivity of 57.5% and a specificity of 100%. According to the EORTC 1999 criteria,  $^{18}\text{F}$ -FDG PET/CT-based treatment response revealed CR in 14 patients ( $^{18}\text{F}$ -FDG PET/CT CR), PR in 11 patients ( $^{18}\text{F}$ -FDG PET/CT PR) and progressive disease in 4 patients ( $^{18}\text{F}$ -FDG PET/CT PD). In terms of  $^{18}\text{F}$ -NaF PET/CT, 4/29 pts (13.8%) had a negative baseline scan, thus failed to depict MM. Regarding the patients, for which a direct lesion-to-lesion comparison was feasible,  $^{18}\text{F}$ -NaF PET/CT depicted 56 of the 129  $^{18}\text{F}$ -FDG positive lesions (43%). Follow-up  $^{18}\text{F}$ -NaF PET/CT showed persistence of 81.5% of the baseline  $^{18}\text{F}$ -NaF positive MM lesions after treatment, despite the fact that 64.7% of them had turned to



<sup>18</sup>F-FDG negative. Treatment response according to <sup>18</sup>F-NaF PET/CT revealed CR in 1 patient (<sup>18</sup>F-NaF PET/CT CR), PR in 5 patients (<sup>18</sup>F-NaF PET/CT PR), SD in 12 patients (<sup>18</sup>F-NaF PET/CT SD), and PD in 7 patients (<sup>18</sup>F-NaF PET/CT PD). Dynamic <sup>18</sup>F-FDG and <sup>18</sup>F-NaF PET/CT studies showed that SUV<sub>average</sub>, SUV<sub>max</sub>, as well as the kinetic parameters K<sub>1</sub>, influx and FD from reference bone marrow and skeleton responded to therapy with a significant decrease (p<0.001). **Conclusion:** <sup>18</sup>F-FDG PET/CT demonstrated a sensitivity of 57.7% and a specificity of 100% in treatment response evaluation of MM. Despite its limited sensitivity, the performance of <sup>18</sup>F-FDG PET/CT was satisfactory, given that 6/9 false negative patients in follow-up scans (66.7%) were clinically characterized as nCR, a disease stage with very low tumor mass. On the other hand, <sup>18</sup>F-NaF PET/CT does not seem to add significantly to <sup>18</sup>F-FDG PET/CT in treatment response evaluation of MM patients undergoing HDT and ASCT, at least shortly after therapy.

## INTRODUCTION

High-dose chemotherapy (HDT) with melphalan followed by autologous stem cell transplantation (ASCT) is the standard of care for multiple myeloma (MM) patients aged 65 years or younger<sup>1,2,3,4,5</sup>. In the last years the incorporation of novel agents (thalidomide, lenalidomide, bortezomib) into induction regimens and maintenance therapy of MM has improved the quality of treatment response, which in turn has led to extended progression free survival (PFS) and overall survival (OS) rates<sup>4,6,7</sup>. This previously unreported, prolonged survival of MM patients renders accurate assessment of response to therapy a necessity. Treatment response evaluation in MM is based on well-defined laboratory parameters and in case of a complete serological response the assessment of plasma cell percentage in bone marrow usually acquired from the iliac crest<sup>8,9</sup>.

<sup>18</sup>F-FDG PET/CT is a sensitive functional imaging modality. The updated International Myeloma Working Group (IMWG) criteria consider patients with focal skeletal lesions and increased uptake with underlying osteolytic destruction in one of the new imaging modalities as indicative of active myeloma<sup>10,11</sup>. Although its routine application in the follow-up of MM is not yet recommended, <sup>18</sup>F-FDG PET/CT appears to be useful in the monitoring of MM and has been proposed to strengthen the evaluation of the quality of treatment response<sup>12,13,14,15,16</sup>.

<sup>18</sup>F-NaF is a PET tracer used for skeletal imaging, which accumulates in both osteoblastic and osteolytic lesions, reflecting regional blood flow and bone remodeling<sup>17,18,19,20</sup>. <sup>18</sup>F-NaF PET/CT is evolving as an important imaging method for the assessment of malignant bone diseases<sup>21,22,23</sup>. Despite being suggested as a potential valuable tool in the assessment of MM<sup>24,25,26,27,28</sup>, three recently published prospective studies have yielded rather discouraging results, regarding the

performance of  $^{18}\text{F}$ -NaF PET/CT in evaluation of myeloma bone disease<sup>29,30,31</sup>.

Nevertheless, the data regarding application of  $^{18}\text{F}$ -NaF PET/CT in MM are still considered to be limited.

The aim of this prospective study was to assess the combined use of the radiotracers  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -NaF in treatment response evaluation of a group of MM patients undergoing HDT followed by ASCT by means of static (whole-body) and dynamic PET/CT (dPET/CT).

## MATERIALS AND METHODS

### Patients

The evaluation included initially 34 patients confirmed to suffer from MM based on the criteria established by the IMWG, at the time point of patient recruitment, and scheduled for treatment with HDT followed by ASCT<sup>32</sup>. All patients had primary disease and had never received chemotherapy. Their mean age was 59.1 years (range 38-73 years). Table 1 presents analytically the characteristics of the patients investigated. Patients with a negative baseline <sup>18</sup>F-FDG PET/CT were excluded from the statistical analysis in order to avoid bias in the interpretation of the results (n=5 patients). Patient data on PFS and OS up to July 2016 (time of writing) are also presented. The analysis was conducted in accordance to the declaration of Helsinki with approval of the ethical committee of the University of Heidelberg and the federal agency of radiation protection.

### PET/CT data acquisition

All patients underwent PET/CT scanning with <sup>18</sup>F-FDG and <sup>18</sup>F-NaF before and after therapy with HDT and ASCT. The mean time between baseline and follow-up study was 95 days (range 47-228 days) (Table 1). The double tracer study in each patient was completed in two consecutive days. For reasons of radiation protection the patients were intravenously administered with a maximum dosage of 250 MBq <sup>18</sup>F-FDG (range 85-246 MBq) on the first day and respectively a maximum dosage of 250 MBq <sup>18</sup>F-NaF (range 167-247 MBq) on the second day. Data acquisition consisted of two parts for each tracer: the dynamic part (dPET/CT studies of the lower lumbar spine and the pelvic skeleton) and the static part (whole body PET/CT). Details regarding data acquisition are described in a previous publication of our group<sup>29</sup>.

## PET/CT data analysis

Data analysis was based on: visual (qualitative) analysis, semi-quantitative evaluation based on SUV calculations, and quantitative analysis of the  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -NaF PET/CT scans, performed before (baseline PET/CT) and after (follow-up PET/CT) treatment.

Qualitative analysis was based on visual assessment of the PET/CT scans, according to criteria applied in previous studies from our group<sup>29, 33</sup>. Briefly, bone marrow/skeletal foci presenting with significantly enhanced  $^{18}\text{F}$ -FDG uptake, for which another benign aetiology was excluded, were considered indicative for myeloma. Afterwards, the results of  $^{18}\text{F}$ -NaF PET/CT were correlated to those of  $^{18}\text{F}$ -FDG PET/CT, which served as reference. The basic concept regarding  $^{18}\text{F}$ -NaF PET/CT evaluation was that only lesions that correlated with respective lesions on  $^{18}\text{F}$ -FDG PET/CT were considered as MM-indicative<sup>29</sup>.

Semi-quantitative evaluation was based on volumes of interest (VOIs) and on subsequent calculation of SUVs. VOIs were drawn with an isocontour mode (pseudo-snake) and were placed over sites of MM involvement as well as over reference tissue<sup>34</sup>. Bone marrow (in the case of  $^{18}\text{F}$ -FDG) and skeleton (in the case of  $^{18}\text{F}$ -NaF) of the 5th lumbar vertebra and os ilium if without focal tracer enhancement served as reference tissue.

Quantitative evaluation of the dynamic  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -NaF PET/CT data, derived from reference tissue of the pelvis, was performed using a dedicated software and based on a two-tissue compartment model, with methods already reported in literature and performed previously from our group<sup>29,35,36,37,38,39,40,41</sup>. The application of a two-tissue compartment model leads to the extraction of the kinetic parameters  $K_1$ ,  $k_2$ ,  $k_3$

and  $k_4$  as well as influx ( $K_i$ ) that describe specific molecular processes for each tracer. In case of  $^{18}\text{F}$ -FDG,  $K_1$  reflects the carrier-mediated transport of  $^{18}\text{F}$ -FDG from plasma to tissue while  $k_2$  reflects the transport of the radiopharmaceutical back from tissue to plasma, and  $k_3$  represents the phosphorylation rate while  $k_4$  the dephosphorylation rate of the glucose analogue. Influx ( $K_i$ ) is derived from the equation  $= (K_1 \times k_3) / (k_2 + k_3)$ . In case of  $^{18}\text{F}$ -NaF, rate constants  $K_1$  and  $k_2$  describe the fluoride ions exchange with hydroxyl groups of hydroxyapatite crystal of the bone and the reverse, while  $k_3$  and  $k_4$  represent the formation of fluoroapatite and the opposite<sup>25</sup>. Influx ( $K_i$ ) is related to  $\text{Ca}^{2+}$  influx and bone apposition rate and, presumably, represents bone remodeling rate<sup>42</sup>.

In addition to performing compartment analysis, a non-compartment model based on the fractal dimension (FD) for the time-activity data was also applied. FD is a parameter of heterogeneity based on the box counting procedure of chaos theory and was calculated for the time activity data in each individual voxel of a VOI. The values of FD vary from 0 to 2 showing the more deterministic or chaotic distribution of the tracer activity via time in a VOI<sup>43</sup>.

### **Treatment response evaluation by laboratory and imaging**

Treatment response evaluation was performed according to the clinical gold standard, based on the European Bone Marrow Transplantation Criteria, introduced by Bladé et al<sup>8</sup> and modified by the IMWG uniform response criteria for multiple myeloma<sup>9</sup>. These criteria served as reference standard in our study.

Treatment response by means of  $^{18}\text{F}$ -FDG PET/CT was assessed according to the European Organization for Research and Treatment of Cancer (EORTC) 1999 criteria leading to four groups of therapy response (complete response,  $^{18}\text{F}$ -FDG PET/CT CR; partial response,  $^{18}\text{F}$ -FDG PET/CT PR; stable disease,  $^{18}\text{F}$ -FDG PET/CT SD; progres-

sive disease,  $^{18}\text{F}$ -FDG PET/CT PD)<sup>44</sup>. Due to lack of defined treatment monitoring criteria based on  $^{18}\text{F}$ -NaF PET/CT, we also applied the EORTC 1999 criteria for this tracer.

Moreover, quantitative data derived from dynamic PET/CT studies from the pelvis were also applied in treatment response evaluation. In particular, the kinetic parameters retrieved from application of two-tissue compartment modelling as well as FD in reference bone marrow or skeleton were compared before and after therapy.

### **Statistical analysis**

Data were statistically evaluated using the STATA/SE 12.1 (StataCorp) software on an Intel Core (2 · 3.06 GHz, 4 GB RAM) running with Mac OS X 10.8.4 (Apple Inc., Cupertino, CA, USA). The statistical evaluation was performed using the descriptive statistics and Wilcoxon rank-sum test. Moreover, we calculated the sensitivity and specificity of  $^{18}\text{F}$ -FDG PET/CT for determination of remission status based on the clinical gold standard<sup>8,9</sup>. The results were considered significant for p less than 0.001 (p<0.001).

## RESULTS

### Treatment response evaluation based on the clinical gold standard

Patient population characteristics, as well as the results of treatment response evaluation are reported in Table 1. All patients showed at least partial clinical response after completion of HDT and ASCT. 5 MM patients had a negative baseline  $^{18}\text{F}$ -FDG PET/CT scan and were, therefore, excluded from the statistical analysis. These 5 patients were also MM-negative on  $^{18}\text{F}$ -NaF PET/CT. Regarding the remaining 29 patients, 3 of them demonstrated complete response (CR) and 26 demonstrated non-CR. In particular, 13 patients showed near complete response (nCR), 4 patients very good partial response (VGPR), and 9 patients showed partial response (PR) according to the clinical evaluation criteria.

### $^{18}\text{F}$ -FDG PET/CT evaluations

Baseline  $^{18}\text{F}$ -FDG PET/CT demonstrated 129 MM-indicative focal lesions in 22 patients. The comparison between  $^{18}\text{F}$ -FDG PET and the underlying low-dose CT findings in these 22 patients revealed 86 circumscribed osteolytic lesions in CT that correlated with the  $^{18}\text{F}$ -FDG avid PET lesions (66.7%). In 5 patients the number of lesions was too large to be exactly calculated (more than 20 lesions). 2 patients demonstrated an intense diffuse pattern of bone marrow uptake without focal lesions. No baseline EMD was detected. After treatment,  $^{18}\text{F}$ -FDG PET/CT became negative in 14 patients, while it remained positive in 15 patients. In correlation with the clinical gold standard,  $^{18}\text{F}$ -FDG PET/CT after therapy was true positive in 15/26 patients with non-CR, and false negative in 11/26 patients with non-CR, resulting in a sensitivity of 57.7%. On the other hand,  $^{18}\text{F}$ -FDG PET/CT was true negative in 3/3 patients with CR, resulting in a specificity of 100%.  $^{18}\text{F}$ -FDG PET/CT demonstrated no false positive results in the skeleton. Two patients demonstrated on follow-up  $^{18}\text{F}$ -FDG



PET/CT pelvic lymphadenopathy and liver lesions. However, after correlation with clinical data, these findings were attributed to inflammatory/post-therapeutic changes and fungus infection respectively. Treatment response evaluation according to the EORTC 1999 criteria revealed 14 patients with CR ( $^{18}\text{F}$ -FDG PET/CT CR), 11 patients with PR ( $^{18}\text{F}$ -FDG PET/CT PR), and 4 patients with PD due to development of new bone marrow lesions ( $^{18}\text{F}$ -FDG PET/CT PD) (Table 2) (Figures 1, 3).

#### **$^{18}\text{F}$ -NaF PET/CT evaluations**

Regarding  $^{18}\text{F}$ -NaF PET/CT evaluations, 4/29 (13.8%) patients failed to depict any MM lesions on the baseline PET/CT. In the remaining patients 108 lesions were demonstrated on baseline  $^{18}\text{F}$ -NaF PET/CT. Follow-up  $^{18}\text{F}$ -NaF PET/CT showed that 88 of the 108 (81.5%) baseline MM-indicative lesions were still  $^{18}\text{F}$ -NaF positive after treatment. In terms of treatment response, 1 patient showed  $^{18}\text{F}$ -NaF PET/CT-CR, 5 patients  $^{18}\text{F}$ -NaF PET/CT-PR, 12 patients  $^{18}\text{F}$ -NaF PET/CT-SD, and 7 patients  $^{18}\text{F}$ -NaF PET/CT-PD (Table 2) (Figures 2, 4).

#### **Comparison between $^{18}\text{F}$ -FDG PET/CT and $^{18}\text{F}$ -NaF PET/CT findings**

In 5 patients with innumerable  $^{18}\text{F}$ -FDG positive lesions,  $^{18}\text{F}$ -NaF PET/CT revealed a more limited disease extent on baseline scan. The 2 patients, who demonstrated an intense diffuse pattern of bone marrow uptake on  $^{18}\text{F}$ -FDG PET/CT, were  $^{18}\text{F}$ -NaF negative. Regarding the 22 patients, for who a direct lesion-to-lesion comparison was feasible,  $^{18}\text{F}$ -NaF PET/CT depicted 56 of the 129  $^{18}\text{F}$ -FDG positive lesions (43%). 57 of the 88 lesions (64.7%) that were still positive on follow-up  $^{18}\text{F}$ -NaF PET/CT had already turned to  $^{18}\text{F}$ -FDG negative, thus were falsely classified as MM-positive by the follow-up  $^{18}\text{F}$ -NaF PET/CT, according to the criteria applied in the study.

#### **Survival data**

1 The data on PFS and OS up to July 2016 (time of writing) are presented in Table 1.  
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3 The follow-up time ranged from 15 to 52 months. Only one patient had died. 12  
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5 patients demonstrated progression, while 18 patients were progression-free. One  
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7 patient was lost to follow-up. Due to lack of late follow-up data for all patients, we  
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9 did not proceed to survival analysis in order to compare the survival rates between the  
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11 different groups.  
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### 13 **Kinetic analysis data**

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16 The results of dPET/CT evaluations from reference tissue before and after therapy are  
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18 presented in Tables 3, 4. In terms of both  $^{18}\text{F}$ -FDG dPET/CT and  $^{18}\text{F}$ -NaF dPET/CT,  
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20 the patients responded to therapy with a statistically significant decrease of the semi-  
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22 quantitative parameters  $\text{SUV}_{\text{average}}$  and  $\text{SUV}_{\text{max}}$ , as well as of the quantitative  
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24 parameters  $K_1$ , influx ( $K_i$ ) and FD ( $p < 0.001$  respectively). The changes in the  
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## DISCUSSION

Assessment of treatment response in MM is based on certain, well-defined laboratory parameters<sup>8,9</sup>. Nevertheless, novel imaging modalities such as PET/CT are nowadays considered valuable tools in improving the definition of response to therapy especially in case of complete response where the percentage of plasma cells in the bone marrow is assessed only from a single location at the iliac crest. Several studies have highlighted the potency of <sup>18</sup>F-FDG PET/CT in accurate response evaluation to therapy in MM<sup>16, 45, 46, 47</sup> while the role of <sup>18</sup>F-NaF PET/CT in the evaluation of myeloma lesions is still being investigated. In the present prospective study we assessed the combined use of <sup>18</sup>F-FDG PET/CT and <sup>18</sup>F-NaF PET/CT in treatment response evaluation of MM patients undergoing HDT with melphalan followed by ASCT.

In patient-based analysis <sup>18</sup>F-FDG PET/CT showed a sensitivity of 57.7%, a result similar to results previously reported by Derlin et al. who found sensitivities of 54.6% and 50.0% for correct determination of remission status after stem cell transplantation using also the same clinical criteria as gold standard<sup>14, 47</sup>. A possible explanation for this relatively limited sensitivity is that MM cells have a rather low proliferation rate and some lesions might be too small to be depicted, given that 6 of the false negative patients (66.7%) were clinically characterized as nCR, a disease stage with very low tumour mass<sup>14, 48</sup>. The phenomenon of achievement of clinical CR with persistence of <sup>18</sup>F-FDG PET/CT positivity after therapy in MM has been studied by Zamagni et al and Bartel et al. These groups have highlighted the fact that MM patients with conventionally defined CR but with persistence of <sup>18</sup>F-FDG PET/CT positive lesions have a higher risk of progression than <sup>18</sup>F-FDG PET/CT negative patients<sup>12, 13</sup>.

Moreover, the Zamagni

group has proven that the achievement of conventional CR and  $^{18}\text{F}$ -FDG PET/CT negativity ensured a significantly prolonged progression free survival (PFS) and an extended overall survival (OS) compared to the achievement of conventional CR but with persistence of  $^{18}\text{F}$ -FDG avidity after therapy<sup>16</sup>. The specificity of  $^{18}\text{F}$ -FDG PET/CT was 100% with 3/3 patients clinically characterized as CR being  $^{18}\text{F}$ -FDG negative.  $^{18}\text{F}$ -FDG PET/CT is considered a relatively specific imaging modality regarding MM response assessment, due to its ability to differentiate between active disease and fibrotic lesions<sup>15,46</sup>. As expected, a direct comparison of the treatment response assessed by the clinical gold standard and  $^{18}\text{F}$ -FDG PET/CT was not feasible.

The performance of  $^{18}\text{F}$ -NaF PET/CT was rather limited. In patient-based analysis, baseline  $^{18}\text{F}$ -NaF PET/CT was negative in 13.8% of the  $^{18}\text{F}$ -FDG PET/CT positive MM patients. Moreover, baseline  $^{18}\text{F}$ -NaF PET/CT depicted only 43% of the  $^{18}\text{F}$ -FDG positive lesions, a result in accordance with a previous study of our group involving 67 MM patients, in which  $^{18}\text{F}$ -NaF PET/CT detected only 39% of the MM lesions demonstrated on  $^{18}\text{F}$ -FDG PET/CT<sup>29</sup>. Regarding follow-up studies,  $^{18}\text{F}$ -NaF PET/CT showed persistence of the majority (81.5%) of the baseline  $^{18}\text{F}$ -NaF positive MM lesions after treatment, despite the fact that 64.7% of them had turned  $^{18}\text{F}$ -FDG negative as a response to HDT and ASCT. The reason for this discordance between  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -NaF PET/CT findings lies on the different molecular mechanisms of the two tracers.  $^{18}\text{F}$ -FDG represents a direct parameter of tumour metabolism. A decline in the tumour  $^{18}\text{F}$ -FDG uptake is expected to be seen with a loss of viable cancer cells, which is in the case in patients with partial or complete response<sup>49</sup>. On the other hand,  $^{18}\text{F}$ -NaF uptake mechanism corresponds to osteoblastic activity. The

1 accumulation of  $^{18}\text{F}$ -NaF in osteolytic lesions, as in case of MM, takes place in the  
2 accompanying, even minimal, reactive osteoblastic changes<sup>19</sup>.  
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4 To date there is little information available about the role of  $^{18}\text{F}$ -NaF PET in treatment  
5 monitoring of systemic cancer therapy. Hillner et al. have recently assessed the impact  
6 of  $^{18}\text{F}$ -NaF PET results in a set of 2,217 oncological patients receiving systemic  
7 therapy. Their results showed a high impact of the modality in patients with  
8 progressive osseous metastatic disease, with a 40% change in treatment plan after  $^{18}\text{F}$ -  
9 NaF PET<sup>21</sup>. The authors stressed, however, the non-tumor specific nature of the tracer  
10 as an indicator of reactive bone formation in response to various insults, and the  
11 limitation of being subject to the flare phenomenon associated with systemic therapy.  
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13 The experience from  $^{99\text{m}}\text{Tc}$ -MDP bone scintigraphy (BS), the analogue of  $^{18}\text{F}$ -NaF  
14 PET for conventional nuclear medicine, is much larger. Although the response to  
15 treatment is evident through a decrease in  $^{99\text{m}}\text{Tc}$ -MDP uptake in BS, several studies  
16 have shown that an increased activity of the bone-seeking tracer in the area of a  
17 tumour lesion may persist for several months after therapy, partly in terms of the  
18 healing, osteoblastic, reactive process<sup>50,51,52,53,54</sup>. Garcia et al. evaluated the combined  
19 use of  $^{99\text{m}}\text{Tc}$ -MDP BS and  $^{18}\text{F}$ -FDG PET in treatment response assessment of bone  
20 metastases in 25 patients suffering from breast and lung cancer. According to their  
21 results, 5 patients with improvement on  $^{18}\text{F}$ -FDG PET scans demonstrated PD and/or  
22 SD on  $^{99\text{m}}\text{Tc}$ -MDP BS. Clinical follow-up, serial tumor markers and radiological  
23 findings confirmed the  $^{18}\text{F}$ -FDG PET findings, leading to the conclusion that some of  
24 the BS results should be interpreted as representing a persistent bone reaction, not  
25 active metastatic disease<sup>55</sup>. This previous experience with bone matrix radiotracers  
26 was the reason that the sensitivity and specificity of  $^{18}\text{F}$ -NaF PET/CT were not  
27 assessed with regard to the clinical gold standard.  
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Apart from the conventional evaluation of whole-body PET/CT scans, we performed quantitative assessment of the dynamic PET/CT data derived from reference bone marrow ( $^{18}\text{F}$ -FDG), and reference skeleton ( $^{18}\text{F}$ -NaF) of the pelvis after application of two-tissue compartment modelling. The quantitative aspect is a major advantage of PET, which is neglected when using whole-body protocols and visual/qualitative evaluation as the only diagnostic tool. Only limited data exist on quantitative assessment of tracer kinetics in MM. Our group has recently shown that the  $^{18}\text{F}$ -FDG kinetic parameters  $K_1$ , influx ( $K_i$ ), as well as SUV from reference bone marrow of the os ilium, correlated significantly with bone marrow malignant plasma cell infiltration rate<sup>40</sup>. The herein presented results revealed that in the case of  $^{18}\text{F}$ -FDG, tracer uptake (reflected by  $\text{SUV}_{\text{average}}$  and  $\text{SUV}_{\text{max}}$ ), its transport capacity ( $K_1$ ), and its influx rate ( $K_i$ ) responded to HDT and ASCT with a significant decrease. These findings are in agreement with previous findings from Dimitrakopoulou-Strauss et al., who studied a group of MM patients undergoing anthracycline-based chemotherapy with dynamic  $^{18}\text{F}$ -FDG PET/CT prior to the onset of therapy and after the first cycle. The authors found a significant decrease ( $p<0.000$ ) of SUV,  $\text{FD}$ ,  $V_B$ , and influx ( $K_i$ ) for  $^{18}\text{F}$ -FDG as derived from reference bone marrow of the os ilium, in response to treatment<sup>56</sup>. The herein presented results provide more evidence in the direction of establishment of  $^{18}\text{F}$ -FDG PET/CT as a tool for treatment response evaluation in MM; we proved that in a group of patients that clinically responded to therapy with at least PR, certain parameters involved in  $^{18}\text{F}$ -FDG metabolism also responded with a significant decrease of their values. Considering that the particular  $^{18}\text{F}$ -FDG parameters correlate with the bone marrow malignant plasma cell infiltration rate, an indicator of myeloma burden and one of the myeloma defining events<sup>10</sup>, our data stress the capacity of  $^{18}\text{F}$ -

FDG dynamic PET/CT to demonstrate bone marrow changes in response to treatment in a molecular level.

Interestingly,  $^{18}\text{F}$ -NaF-associated kinetic parameters demonstrated similar changes as  $^{18}\text{F}$ -FDG in response to therapy. In particular,  $^{18}\text{F}$ -NaF uptake ( $\text{SUV}_{\text{average}}$  and  $\text{SUV}_{\text{max}}$ ), the rate of fluoride ions exchange with hydroxyl groups of hydroxyapatite crystal of the bone ( $K_1$ ),  $\text{Ca}^{2+}$  influx, bone apposition rate and, presumably, bone remodelling rate ( $K_i$ ) decreased significantly after HDT and ASCT. Myelomatous bone disease after ASCT is little understood<sup>57</sup>. The fact that bone marrow transplantation may affect the skeleton has been demonstrated by Gandhi et al. in an heterogeneous group of oncological patients, one of which suffered from MM. The authors showed that 3 months after ASCT there was a significant decline of bone mineral density in the femoral neck and a non-significant trend towards reduction in the lumbar spine<sup>58</sup>. Terpos et al. have shown that bone formation markers do not normalise until the eighth month post-ASCT, providing an indication that bone formation may delay in normalising<sup>59</sup>. Further, in one of the few published treatment monitoring studies by means of dynamic  $^{18}\text{F}$ -NaF PET, Installé et al. have demonstrated in 14 patients with Paget's disease receiving bisphosphonates therapy that  $\text{SUV}_{\text{max}}$ ,  $K_1$ , and influx constant  $K_i$  decreased significantly as response to treatment<sup>60</sup>. To the best of our knowledge, these are the first data regarding bone turnover changes in MM patients receiving HDT and ASCT, evaluated by means of dynamic PET/CT.

In addition to two-tissue compartment modelling for tracer kinetics assessment, we also applied a non-compartmental approach based on the box counting procedure of the chaos theory for the analysis of dPET data, resulting in another index representative of tissue heterogeneity, fractal dimension (FD)<sup>43</sup>. Fractal geometry has

1 found use in pathology for assessment of irregularities<sup>61</sup>. Our group has shown that  
2 FD of <sup>18</sup>F-FDG correlates significantly with the degree of bone marrow malignant  
3 plasma cell infiltration rate<sup>40</sup>. In the present study we found that FD for both tracers  
4 decreased significantly, reflecting a decline of the heterogeneity of the concentration  
5 of both tracers over time in response to treatment, a result in accordance with the  
6 changes of compartment-derived kinetic parameters.  
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14 This study has some limitations. Firstly, the number of patients enrolled was relatively  
15 small. Therefore, further studies with a larger study population are warranted to  
16 generalize the herein presented results. Secondly, most of the PET/CT positive  
17 findings were not histopathologically confirmed. However, this is usually not possible  
18 in the clinical setting. Another limitation is the confinement of the dynamic PET/CT  
19 studies only in the anatomic area of the pelvis, since whole-body dynamic studies  
20 cannot be performed. We used a two-bed position protocol for the dynamic PET  
21 acquisition, which allows the study of a relatively large field of view of 44 cm.  
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24 Nevertheless, new PET/CT scanners allow dynamic studies over several bed positions  
25 by using a continuous bed movement, thus, facilitating the use of dynamic protocols  
26 and reducing the whole acquisition time. Finally, the lack of late follow-up data for all  
27 patients prevented us from proceeding to survival analysis between the different  
28 patient groups, which will be the topic of a future publication of our group.  
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## CONCLUSION

In the present study  $^{18}\text{F}$ -FDG PET/CT demonstrated a sensitivity of 57.7% and a specificity of 100% in treatment response evaluation of 29 MM patients undergoing HDT and ASCT, using the clinical response criteria as reference standard. Despite its limited sensitivity, the performance of  $^{18}\text{F}$ -FDG PET/CT was satisfactory, given that 6/9 false negative patients in follow-up scans (66.7%) were clinically characterized as nCR, a disease stage with very low tumor mass. In contrary,  $^{18}\text{F}$ -NaF PET/CT did not aid significantly in treatment response evaluation of MM patients, at least in an early phase. Dynamic PET/CT studies demonstrated a decrease of SUVs and specific kinetic parameters in reference tissue for both  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -NaF as response to treatment, reflecting changes in a molecular level before any morphological changes take place.

## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Ethical approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Furthermore, the study was approved by the Ethical Committee I of the University of Heidelberg and the Federal Radiation Protection Agency.

**Informed consent:** Informed consent was obtained from all individual participants included in the study.

**Acknowledgment:**

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## FIGURE LEGENDS

**Figure 1:** A 70-years old stage III MM patient scheduled for HDT and ASCT, undergoing  $^{18}\text{F}$ -FDG PET/CT before and after therapy. Maximum intensity projection (MIP)  $^{18}\text{F}$ -FDG PET/CT before therapy (left) revealed a mixed pattern of  $^{18}\text{F}$ -FDG uptake with intense, diffuse uptake in the axial skeleton and multiple, focal bone marrow lesions. Follow-up  $^{18}\text{F}$ -FDG PET/CT MIP three months after ASCT (right) demonstrated a complete remission of both diffuse bone marrow uptake as well as focal myeloma-indicative lesions ( $^{18}\text{F}$ -FDG PET/CT-CR).  $^{18}\text{F}$ -FDG uptake in cervical, abdominal and inguinal lymph nodes in the follow-up scan was attributed to inflammatory reaction after therapy, thus considered benign. Response according to clinical criteria was CR and according to the  $^{18}\text{F}$ -FDG PET EORTC criteria also CR.

**Figure 2:** Whole body  $^{18}\text{F}$ -NaF PET/CT MIP before and after therapy of the same patient as in figure 1. Baseline  $^{18}\text{F}$ -NaF PET/CT (left) demonstrated several  $^{18}\text{F}$ -NaF positive skeletal lesions, which partly corresponded to respective lesions on  $^{18}\text{F}$ -FDG PET/CT (Fig. 1) and were considered myeloma-indicative, as well as several degenerative changes mostly in the spine. Follow-up  $^{18}\text{F}$ -NaF PET/CT MIP after therapy (right) showed remission of some of the MM-indicative lesions but at the same time persistence of several of them ( $^{18}\text{F}$ -NaF PET/CT-PR). Response according to clinical criteria was CR and according to the  $^{18}\text{F}$ -NaF PET criteria applied in our study PR.

**Figure 3:** A 68-years old stage III MM patient scheduled for HDT and ASCT undergoing  $^{18}\text{F}$ -FDG PET/CT before and after therapy. Transaxial  $^{18}\text{F}$ -FDG PET/CT in the cervical level before therapy (upper row) revealed an  $^{18}\text{F}$ -FDG

1 avid, MM-indicative lesion in the transverse process of the 4<sup>th</sup> cervical  
2 vertebrae. The patient underwent a follow-up <sup>18</sup>F-FDG PET/CT 49 days after  
3 ASCT (lower row), which demonstrated complete metabolic remission of the  
4 MM lesion. According to the EORTC 1999 criteria, the patient was  
5 characterized as <sup>18</sup>F-FDG PET/CT-CR. According to clinical criteria, the  
6 patient's response was nCR.  
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14 **Figure 4:** Transaxial <sup>18</sup>F-NaF PET/CT before and after therapy of the same  
15 patient as in figure 3. Baseline <sup>18</sup>F-NaF PET/CT (upper row) revealed also the  
16 <sup>18</sup>F-FDG avid, myeloma-indicative lesion in the transverse process of the 4<sup>th</sup>  
17 cervical vertebrae as <sup>18</sup>F-NaF positive. In contrary to <sup>18</sup>F-FDG PET/CT (Fig.3),  
18 the lesion demonstrated a persistence of the <sup>18</sup>F-NaF accumulation in the  
19 follow-up <sup>18</sup>F-NaF PET/CT (lower row) after HDT and ASCT (<sup>18</sup>F-NaF  
20 PET/CT-SD).  
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32 **Figure 5:** Time activity curves (TACs) depicting <sup>18</sup>F-FDG concentration  
33 during the 60 minutes of dynamic PET acquisition in reference bone marrow  
34 before (upper row) and after therapy with HDT and ASCT (lower row). The  
35 curves are derived from bone marrow of the os ilium that served as reference  
36 (blue curve with green dots) and from the common iliac artery (curve with  
37 gold dots). Small decrease in the radiotracer concentration in reference tissue  
38 VOIs after therapy. The corresponding kinetic parameters responded to therapy  
39 also with a decrease.  
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52 **Figure 6:** Time activity curves (TACs) depicting <sup>18</sup>F-NaF concentration  
53 during the 60 minutes of dynamic PET acquisition in reference skeleton before  
54 (upper row) and after therapy with HDT and ASCT (lower row). The curves  
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are derived from osseous tissue of the os ilium that served as reference (blue  
curve with green dots) and from the common iliac artery (curve with gold  
dots). Decrease in the radiotracer concentration in reference tissue VOIs after  
therapy. The corresponding kinetic parameters responded to therapy also with  
a decrease.

**Table 1** Characteristics, treatment response and survival rates of the patients investigated in the study.

Patient number	Stadium	Age	Gender	Time between ASCT-follow-up PET (days)	Clinical response	<sup>18</sup> F-FDG PET/CT response	<sup>18</sup> F-NaF PET/CT response	Progression after therapy	PFS (months)	OS (months)
1	1	66	F	78	PR	PR	Negative baseline scan	Yes	39	44
2	3	65	M	228	CR	CR	PR	Yes	16	49
3	3	57	M	117	nCR	PR	SD	Yes	17	32
4	3	70	M	97	CR	CR	PR	Yes	30	43
5	3	73	M	132	nCR	PR	SD	No	37	37
6	1	53	M	64	PR	CR	SD	No	31	31
7	3	46	F	81	nCR	CR	SD	Yes	26	35
8	3	53	F	83	VGPR	PR	SD	Lost to follow-up		
9	3	69	F	52	PR	CR	CR	Yes	4	41
10	3	49	M	120	nCR	CR	Negative baseline scan	No	28	28
11	3	43	M	93	nCR	PD	PD	No	28	28
12	3	60	M	67	nCR	PR	PD	Yes	9	18 (dead)
13	3	68	M	75	nCR	PR	PR	Yes	25	25
14	3	69	M	101	nCR	CR	SD	No	28	28
15	3	68	F	49	nCR	CR	SD	No	24	24
16	3	53	M	70	PR	CR	SD	No	26	26
17	3	62	F	145	nCR	PD	PD	Yes	20	27
18	3	70	M	51	VGPR	PR	PD	No	33	33
19	1	60	M	115	PR	PR	SD	No	24	24
20	3	59	F	47	VGPR	PR	SD	No	23	23
21	3	60	M	86	CR	CR	SD	No	52	52
22	1	63	F	106	nCR	CR	Negative baseline scan	No	23	23
23	3	59	F	120	PR	CR	SD	Yes	18	24
24	3	48	M	102	nCR	CR	PR	No	18	18
25	3	61	M	89	PR	PR	Negative baseline scan	No	20	20
26	3	45	M	67	PR	PD	PD	No	20	20
27	3	71	M	125	PR	CR	PR	Yes	18	19
28	3	59	F	128	nCR	PR	PD	Yes	7	19
29	3	62	F	55	VGPR	PD	PD	No	15	15
30	1	62	M	105	VGPR	Negative baseline scan (excluded)	Negative baseline scan			
31	3	69	F	136	VGPR	Negative baseline scan (excluded)	Negative baseline scan			
32	3	38	M	48	VGPR	Negative baseline scan (excluded)	Negative baseline scan			

33	3	46	M	98	nCR	Negative baseline scan (excluded)	Negative baseline scan			
34	3	54	M	86	nCR	Negative baseline scan (excluded)	Negative baseline scan			

M, male; F, female; CR, complete response; nCR, near complete response; VGPR, very good partial response; PR, partial response; SD, stable disease; PD, progressive disease.

All patients were alive at the time of writing, with the exception of patient no 12.

**Table 2** Treatment response of the 29 MM patients according to clinical criteria,  $^{18}\text{F}$ -FDG PET/CT criteria and  $^{18}\text{F}$ -NaF PET/CT criteria.

Clinical response	$^{18}\text{F}$ -FDG PET/CT response	$^{18}\text{F}$ -NaF PET/CT response
CR= 3 patients nCR= 13 patients VGPR= 4 patients PR= 9 patients	CR= 14 patients PR= 11 patients PD= 4 patients	CR= 1 patient PR= 5 patients SD= 12 patients PD= 7 patients

CR, complete response; nCR, near complete response; VGPR, very good partial response; PR, partial response; SD, stable disease; PD, progressive disease

**Table 3** Descriptive statistics of mean and median values prior and after HDT and ASCT for the  $^{18}\text{F}$ -FDG semi-quantitative and quantitative parameters in reference bone marrow. The values of parameters  $K_1$ ,  $k_2$ ,  $k_3$ ,  $k_4$  and influx are 1/min. SUVs and FD have no unit.

Parameter	Mean prior	Median prior	Mean after	Median after
SUV <sub>average</sub> *	2.2	1.9	1.6	1.3
SUV <sub>max</sub> *	3.9	3.3	2.5	2.0
$K_1$ *	0.214	0.186	0.158	0.144
$k_2$ *	0.710	0.693	0.570	0.571
$k_3$	0.054	0.050	0.050	0.045
$k_4$	0.025	0.020	0.028	0.020
influx*	0.014	0.013	0.011	0.009
FD*	1.146	1.138	1.086	1.065

\*significant probabilities ( $p < 0.001$ )

**Table 4** Descriptive statistics of mean and median values prior and after HDT and ASCT for the  $^{18}\text{F}$ -NaF semi-quantitative and quantitative parameters in reference skeleton. The values of parameters  $K_1$ ,  $k_2$ ,  $k_3$ ,  $k_4$  and influx are 1/min. SUVs and FD have no unit.

Parameter	Mean prior	Median prior	Mean after	Median after
SUV <sub>average</sub> *	8.7	8.4	6.9	6.3
SUV <sub>max</sub> *	14.6	13.8	10.5	10.0
$K_1$ *	0.200	0.177	0.143	0.116
$k_2$	0.413	0.421	0.329	0.272
$k_3$	0.279	0.249	0.250	0.229
$k_4$	0.015	0.013	0.013	0.012
influx*	0.076	0.070	0.059	0.054
FD*	1.382	1.390	1.340	1.342

\*significant probabilities ( $p < 0.001$ )

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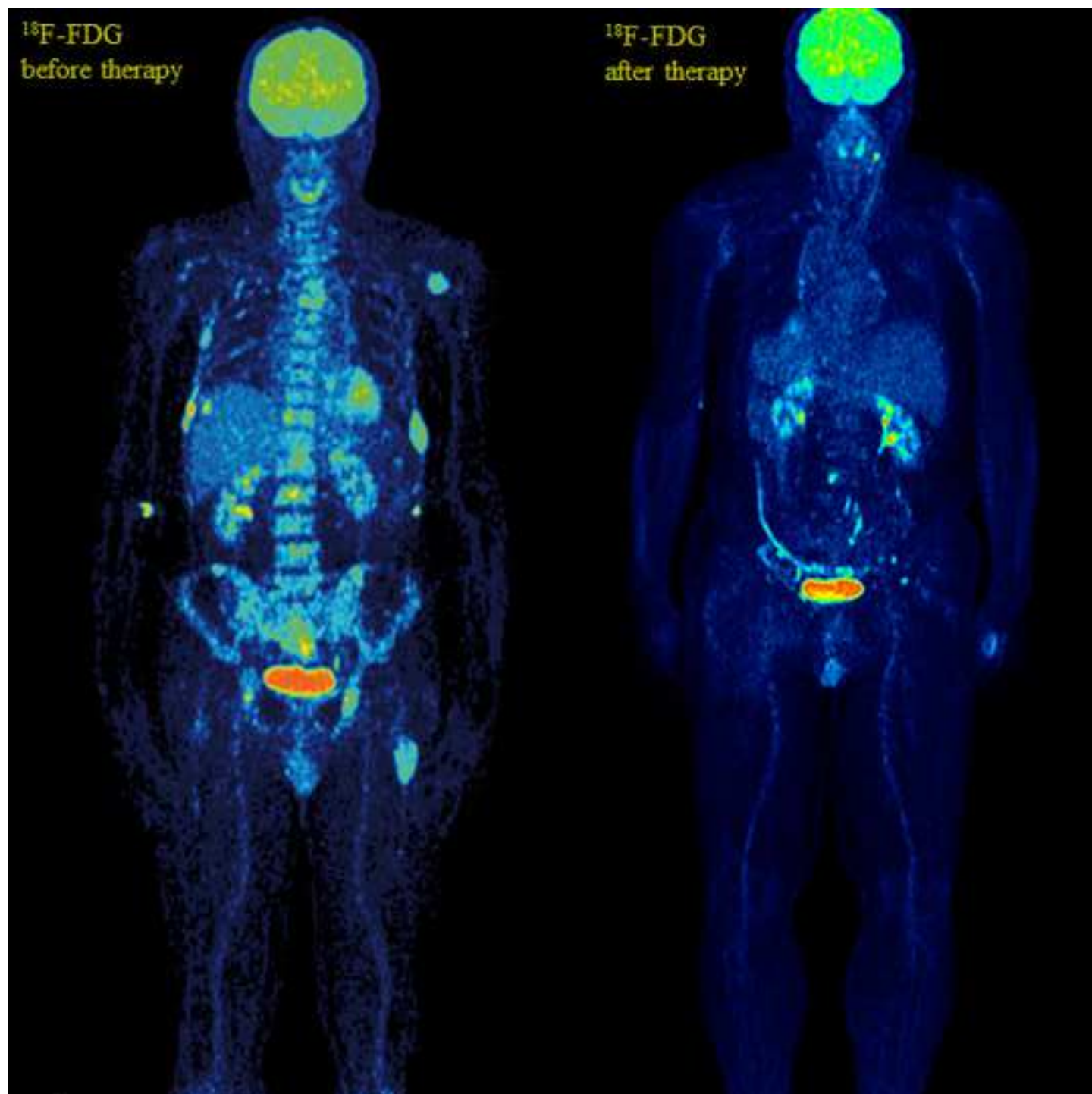
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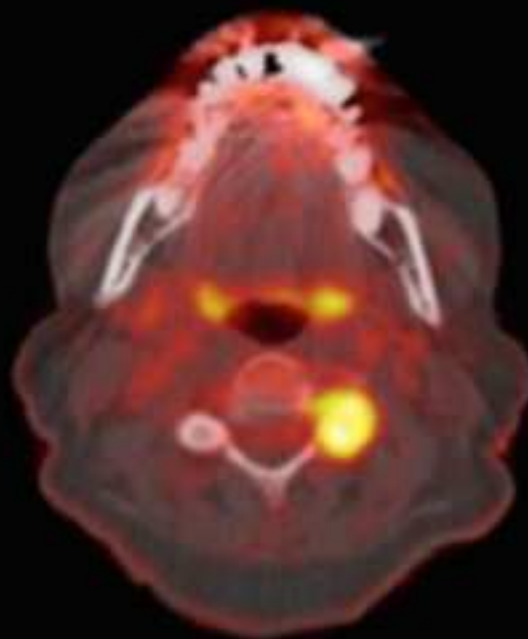
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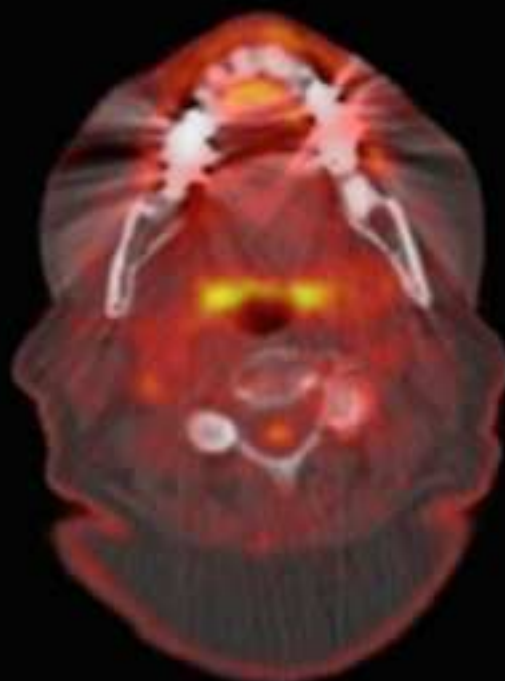




$^{18}\text{F}$ -FDG  
before therapy



$^{18}\text{F}$ -FDG  
after therapy





$^{18}\text{F}$ -NaF  
before therapy



$^{18}\text{F}$ -NaF  
after therapy

